

present invention displays increased sensitivity in the analysis of protein mixtures over a typical MALDI time-of-flight mass spectrometry experiment. To compare the two methods a two component mixture consisting of a tryptic digest of bovine hemoglobin α and β was analyzed on the apparatus of the present invention and on a state of the art high resolution time-of-flight mass spectrometer. The ion mobility experiment, for which the 3-dimensional plot of mass spectra is shown in **FIG. 24**, observed a greater percentage of the total amino acids present in the sample relative to the optimized MALDI-TOF protocol (94% amino acid coverage for both hemoglobin α and β versus 75% and 68% on the MALDI-TOF instrument). The observed increase in % coverage is attributed to the increased sensitivity of the present invention. As a further test a more complex mixture consisting of horse heart cytochrome c, chicken egg white lysozyme, bovine serum albumin, bovine hemoglobin α and bovine hemoglobin β was used. The same sample was analyzed using optimized sample preparation procedures on both the apparatus of the present invention and the MALDI-TOF instrument. The table shown in **FIG. 25** clearly illustrates that the apparatus of the present invention yields higher overall % amino acid coverage and individual % amino acid coverage for a complex protein mixture. In addition, the apparatus of the present invention demonstrates higher sensitivity toward lysine terminated digest fragments. (Krause, E. et al. *Anal. Chem.* 1999, 71, 4160-4165). This phenomenon is typified in the case of cytochrome c, for which both experiments result in 60% of the total predicted arginine terminated fragments being observed, but the experiment using the apparatus of the present invention results in the observation of a much greater percentage of the lysine terminated fragments (52% versus 16%). The results suggest that performing MALDI/ion Mobility/Mass analysis of protein mixtures where ions are formed in a low pressure environment (is this case 5 Torr helium) involves a different desorption process than when ions are formed by MALDI in a high vacuum environment. This statement is further supported by a comparison of spectra obtained in the two environments. If the same digest sample is analyzed with the high vacuum instrument using the same matrix and sample preparation as with the apparatus of the present invention the % coverage for a protein digest or a digest of a complex protein mixture is extremely low and only a few fragments are identifiable.

[0110] Therefore, further objects of the present invention include simplification, increased throughput, increased overall sensitivity, and increased sensitivity toward lysine terminated digest fragments present in complex mixtures.

REFERENCES

- [0111] All patents and publications mentioned in the specification are indicative of the level of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.
- [0112] U.S. Pat. No. 4,390,784 6/1983 Browning, et al.
- [0113] U.S. Pat. No. 4,855,595 8/1989 Blanchard
- [0114] U.S. Pat. No. 5,235,182 8/1993 Avida et al.
- [0115] U.S. Pat. No. 5,189,301 2/1993 Thekkadath
- [0116] U.S. Pat. No. 5,736,740 4/1998 Franzen
- [0117] U.S. Pat. No. 5,905,258 5/1999 Clemmer et al.
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- [0141] One skilled in the art readily appreciates that the present invention is well adapted to carry out the objectives and obtain the ends and advantages mentioned as well as those inherent therein. Systems, methods, procedures and techniques described herein are presently representative of the preferred embodiments and are intended to be exemplary and are not intended as limitations of the scope. Changes